PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Kousuke TANI, *et al.*

Docket No: Q83408

Appln. No.: 10/506,536

Group Art Unit: 1626

Confirmation No.: 1208

Examiner: Chung, Susannah Lee

Filed: September 03, 2004

For: 8-AZAPROSTAGLANDIN DERIVATIVE COMPOUNDS AND DRUGS CONTAINING THE COMPOUNDS AS ACTIVE INGREDIENT

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Tsutomu Shiroya, hereby declare and state:

THAT I am a citizen of Japan;

THAT I have received the degree of a Master of Science in 1989 from Kyoto Pharmaceutical University;

THAT I have been employed by Ono Pharmaceutical Co., Ltd. since 1989, where I hold a position as HEAD of pharmacology Group 3, with responsibility for study of prostaglandin at the Research Institute of Ono Pharmaceutical Co., Ltd.;

THAT this declaration is made for support of the above identified U.S. Patent Application;

THAT I am a co-inventor of the present application; and

THAT the following experiments (Activity of EP_2 Receptor Agonist) were conducted under my direct supervision.

EP_2 Receptor Binding Activity

Experiment for measurement of the an EP_2 receptor binding activtity with the cells expressing prostanoid receptor sub-types

According to the method of Sugimoto *et al.* (*J. Biol. Chem.*, 267, 6463-6466 (1992)), CHO cells which expressed prostanoid receptor sub-types (murine EP_1, EP_2, $EP_{3\alpha}$, and EP_4, respectively) were prepared and used as membrane authentic samples.

A reaction solution (200 μl) containing the prepared membrane fraction (0.5 mg/ml) and ^{3}H-PGE_2 was incubated at room temperature for 1 hour. The reaction was terminated with ice cold buffer (3 ml), and the reaction mixture was filtered under suction through a glass filter (GF/B), on which the binding ^{3}H-PGE_2 was trapped. The binding radioactivity was measured by means of a liquid scintillator.

The Kd value was obtained from the Scatchard plots [*Ann. N.Y. Acad. Sci.*, 51, 660 (1949)]. Non-specific binding was obtained as the binding in the presence of an excess amount (2.5 μM) of unlabelled PGE_2. Measurement of the binding inhibition for ^{3}H-PGE_2 with the compounds of the present invention was performed by adding ^{3}H-PGE_2 (2.5 nM) and a series of concentrations of the compound of the present invention. In this reaction, the following buffer was used in all cases.

Buffer: 10 mM potassium phosphate (pH 6.0), 1 mM EDTA, 10 mM $MgCl_2$, and 0.1M NaCl.

Dissociation constant Ki (μM) of each compound was calculated from the following equation.

$Ki = IC_{50}/(1+([C]/Kd))$

IC_{50} : The concentration of the compound of the present invention which inhibits half of the specific binding of [^{3}H]PGE_2

C : The concentration of [^{3}H]PGE_2

Kd : The dissociation constant of [^{3}H]PGE_2

The results are shown below.

Example No.	Ki(nM)
	EP2
6(32)	0.5
6(48)	3.5
6(53)	1.0
6(60)	0.4
6(63)	0.4
6(74)	1.5
6(77)	0.4
6(89)	0.6

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: August 30, 2007

Tsutomu Shiroya

Tsutomu Shiroya